EXHIBIT 17

Amendment As A Submission Under 37 C.F.R. § 1.114(c)

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Applicants: Michael Wayne Graham et al.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Michael Wayne Graham and Robert Norman Rice

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For : SYNTHETIC GENES AND GENETIC CONSTRUCTS

30 Rockefeller Plaza
20th Floor
New York, NY 10112

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF DR. ARTHUR RIGGS

I, Dr. Arthur D. Riggs, declare as follows:

1. I am a Professor of Biology, Chair of the Department of Diabetes and Metabolic Diseases Research and Director Emeritus at the Beckman Research Institute of City of Hope, in Duarte, California. A copy of my *curriculum vitae* and a list of my publications are attached hereto as **Exhibit A**.

2. I have been retained by the Assignee's counsel as a technical expert in connection with the above-identified application. I am being compensated at $600.00 per hour (or a maximum of $5,000 for a day). I understand that a licensee of the subject application has a research agreement with certain investigators at the City of Hope. However, I am not otherwise affiliated with the Assignee or the licensee.

I. Scope of Opinion

3. I have been provided with, and asked to review, the Australian Provisional Patent Application No. PP2492 ("Applicants' Provisional"), the claims of U.S. Application 10/759,841 amended as indicated in **Exhibit B** hereto, the Final Office Action dated January 22, 2009, and the references cited within that Final Office Action, including the Fire et al. Provisional Patent Application No. 60/068,562 ("Fire Provisional"). I have also been provided with, and asked to review, U.S. Patent No. 5,580,767, issued December 3, 1996 ("Cowsert et al."), PCT International Publication No. WO 94/01550, published January 20, 1994 ("Agrawal et al."), and U.S. Patent No. 5,514,546, issued May 7, 1996 ("Kool").

4. I have been asked to provide my opinions of what would have been the views of a person of ordinary skill in the art as of March 1998. I believe I can accurately describe the perspective of such a person. In March 1998, I was actively involved in the areas of gene regulation and epigenetic mechanisms. For the purpose of this declaration I have understood that one skilled in this art in 1997 or 1998 would have at least a doctoral degree, e.g. a Ph.D. degree, in molecular biology or a related discipline, have post-doctoral training, have knowledge in cell biology, biochemistry, developmental biology and genetics, and be well trained in laboratory methodologies.

5. The opinions set forth in this declaration are based on my professional knowledge and expertise, as indicated in my *curriculum vitae*, my review of the Applicants' Provisional Application, and the Final Office Action dated January 22, 2009, including the documents cited in the Final Office Action, as well as additional documents cited in this declaration.

II. Disclosure of the Cited Prior Art

6. The Fire et al. Provisional is based on and incorporates the results published in the 1998 publication in Nature ("Fire et al. Nature publication") where RNA interference (RNAi) was first described in *C. elegans* (Fire et al, Nature, 1998, attached hereto as **Exhibit C**). When I first heard the results, I, along with many of my colleagues, was astonished that the injection of double-stranded RNA had such an effect on gene expression. In that publication, Fire et al. described the exogenous delivery of double-stranded RNA by injecting the RNA into the body cavity or gonads of *C. elegans*. Thus, the RNA was added extracellularly. Yet, as Fire et al. noted, "dsRNA-mediated interference showed a surprising ability to cross cellular boundaries" in *C.elegans*. Fire et al. also mention that injection into the cytoplasm of intestinal cells is effective, but no data supporting this methodology was reported. The double-stranded RNA was produced *in vitro* as separate strands, subsequently annealed, and was sequence specific for the target genes, covering lengths of 299 to 1033 nucleotides of the target genes. Injection of these molecules resulted in a silencing effect of the target gene expression that was one-hundred times more efficient than that observed by injecting antisense RNA alone, as evidenced by the fact that Fire et al. witnessed inhibition of gene expression with as little as "a few molecules [of double-stranded RNA] per cell." The observed gene silencing correlated with a decrease or elimination of the target mRNA, yet the mechanism by which the double-stranded RNA was causing this effect was unknown. Based on his observations, Fire et al. did indicate that "[a] simple antisense model is not likely: annealing between a few injected RNA molecules and excess endogenous transcripts would not be expected to yield observable phenotypes" (Fire et al., Nature, 1998). In the Fire et al. Provisional, Fire et al. confirm that their "invention differs from antisense-mediated interference in both approach and effectiveness."

7. Reviewing the disclosure of the Fire et al. Provisional Application, it was evident to me that Fire et al. included several generalized extensions of his RNA interference discovery, most likely due to a lack of mechanism to describe the results that he had observed. For example, although Fire et al. Provisional Application mentions mammalian cells, the experimental data reported would not have motivated one skilled in the art to add 299 to 1033 base-pair double-stranded RNA to the cytoplasm of mammalian cells because it was known in that art that these cells mounted a non-specific response to double-stranded RNA that would globally inhibit gene expression. The Fire et al. Provisional purports that all of the readily conceivable molecular biology modifications would be tolerated by the experimental system of Fire et al. If one of ordinary skill in the art considered all of the choices offered by the Fire et al. Provisional, a very large number of permutations are apparent. Confounding an attempt to select any permutation is the lack of a clear preference or suggestion, other than in the examples, for one combination of elements over another. While numerous combinations are disclosed as possible, there is no disclosure of which combinations other than the exemplified combinations, would work. Thus, aside from knowing that the technique as exemplified would work in *C. elegans*, one of skill in the art would be no closer to successfully modifying the technique after reading the Fire et al. Provisional than they would have been if they simply read the Fire et al. Nature publication. Moreover, if one looked to the references in the art at the time, they would also fail to find relevant guidance because practically nothing else was known about the then very new observations reported by Fire et al.

8. One might have come across antisense RNA technology, which was another approach to gene silencing that was technically different from interference described by Fire et al. The Examiner has cited Cowsert et al. and Agrawal et al. from the antisense art to argue that the Applicants' invention was obvious at the time. However, it was clear that the interference

described by Fire et al. was not working via an antisense mechanism, thus invalidating the theoretical parallel drawn by the Examiner. Antisense technology required the hybridization of the antisense molecule with its target DNA or mRNA and was highly dependent on the concentration of antisense molecules in the cells. Cowsert et al. describe the use of chemically synthesized antisense oligonucleotides to inhibit the function of influenza virus RNA. Agrawal et al. describe the design and use of small chemically synthesized antisense oligonucleotides and their effectiveness for inhibiting gene expression. In my opinion, these references do not offer any teaching that one of ordinary skill in the art at the time could have applied to predictably modify the teachings of Fire et al. to produce the Applicants' invention.

9. It is my opinion that the teachings of Kool are irrelevant to an invention from the RNA interference art. Kool describes the use of stem-loop oligonucleotides as hybridization probes to identify nucleic acids. Kool also teaches that the stem-loop oligonucleotides could be used to denature double-stranded target nucleic acids by forming triplex structures. Based on the teaching of Fire et al. one of ordinary skill in the art would know that the oligonucleotides taught by Kool would not have functioned in RNA interference because their targeting regions were located in the single-stranded portions of the molecules and their double-stranded stem regions were short. In my opinion, Kool does not offer any teaching that one of ordinary skill in the art at the time could have applied to modify the teachings of Agrawal et al. to produce the Applicants' invention.

III. Interpretation of the Claims

10. My interpretation of the claims attached hereto as **Exhibit B** is based on my understanding of how one of ordinary skill in the art would have understood the terms appearing in the claims in the context of the claims as a whole, in view of the description of the invention set forth in the patent.

11. In general terms, the claims relate to double-stranded DNA constructs, or mammalian cells, tissues or organs containing such constructs, which are used to reduce target gene expression in mammalian cells. More specifically, claim 172 describes in detail the aspects of a double-stranded DNA construct that has a single promoter that is operable in mammalian cells, which drives the transcription of a single DNA sequence that has three sub-sequences: (1) a sequence ("structural gene sequence") that has 20-30 consecutive nucleotides that are identical to the sequence of a target gene; (2) an intervening sequence or "stuffer fragment" distinct from either sub-sequence (1) or (3); and (3) a sequence that is the inverted repeat of the first sub-sequence ("20-30 consecutive nucleotides identical in sequence to, and in an inverted orientation relative to, the 20-30 nucleotides of the first structural gene sequence"). It is my understanding that the transcription of this construct would, in a cell, result in a single RNA strand that forms a hairpin structure within which sub-sequence corresponding to (1) and sub-sequence corresponding to (3) are in a duplexed state and the distinct stuffer (sub-sequence (2)) forms a loop at one end of the hairpin. It is also my understanding that the loop formed by the stuffer allowed for complete intramolecular base-paring between sub-sequence (1) and sub-sequence (3). Following transfection of the DNA construct into mammalian cells, the DNA construct will be transcribed in the nucleus and the produced RNA hairpin would be capable of repressing or reducing expression of the target gene.

12. A number of dependent claims further specify the details of the double-stranded DNA construct described in claim 172. Claim 176 further specifies the exon as the region of a gene to be targeted. Claims 177 to 180 further specify types of possible target genes for the DNA construct. Claim 181 to 183 further specifies the length of the stuffer fragment that separates the structural gene sequences within the DNA construct of claim 172. Claim 184 further specifies the total length of the double-stranded DNA construct. Claims 185 and 186 further specify methods to introduce the double-stranded DNA construct into mammalian cells. Claim 187 further specifies that the DNA construct of the process of claim 172 is integrated into the genome of the targeted mammalian cell.

13. Independent claim 188 describes a mammalian cell having the double-stranded DNA construct of claim 172 and independent claim 200 describes a mammalian cell, tissue, or organ having the double-stranded DNA construct of claim 172. A number of dependent claims further specify the type of target genes, the size of the stuffer fragment, and the incorporation of the DNA construct into the genome of the mammalian cell, as discussed above for the claims dependent on claim 172.

IV. Obviousness Rejections

14. I have read the Examiner's arguments in the January 22, 2009 Final Office Action offered in support of the obviousness rejection of the Applicants' claimed invention over Fire et al. Patent taken with Cowsert et al. or over Agrawal et al. in view of Kool and Cowsert et al., and I respectfully disagree with the Examiner's conclusions. While it is clear that Fire et al. described the use of double-stranded RNA to inhibit gene expression, the methods by which they did so significantly differ from those claimed by Applicants and provide no evidence that would predict the success of the Applicants' invention in March 1998. Additionally, the combination of

references from the antisense field and the triplex-forming oligonucleotide field would not have aided one of skill in the art in developing a successful DNA construct for RNA interference.

15. At the time of the Fire et al. Nature publication, those skilled in the art were mystified by what Fire et al. had described. Since such an experimental system had never been reported before, the only knowledge in the art was derived from the Fire et al. Nature publication. Therefore, it was impossible to predict how the results would be affected by changes made to the experimental system reported by Fire et al. At that time, numerous issues concerning perturbations to Fire's system would be readily apparent and be a cause for concern to one of ordinary skill in the art, including:

(1) How would changes in the delivery technique of the RNA affect the results?

(2) How would changes to the RNA secondary structure affect the results?

(3) How would changes to the size of the double stranded RNA affect the results?

(4) How would endogenous production of double-stranded RNA affect mammalian cells?

As one considers the above questions, numerous variables become apparent that could impact the ability of a RNA molecule to promote the effects reported by Fire et al. These same variables would have prevented one from predicting the effects of the proposed changes. I describe a number of these variables below.

Changes in the delivery technique of the RNA

16. Fire et al. delivered RNA that had been synthesized *in vitro* to target cells via injection. It was known in the art that a certain level of delivery efficiency was associated with injecting nucleic acid molecules into cells or into the body cavity of *C. elegans*. It was also known that the efficiency of introducing nucleic acid molecules varied amongst different

introduction techniques, such as electroporation, lipid-mediated carrier transport or chemical-mediated transport. It was unknown at the time whether the effects observed by Fire et al. would have been obtained if one used one of these other methods of introducing double-stranded RNA into the cells.

17. As an extension of the delivery question, one may have asked if the double-stranded RNA could have been produced in the cell and, if so, how. Intracellular production could happen in the cytoplasm, via various viral vectors, or it could happen in the nucleus, where most RNA production occurs. Producing RNA in the nucleus presents a number of additional unknowns that would have to be resolved to recapitulate the original scenario demonstrated by Fire et al., *i.e.* double-stranded RNA introduced to the cytoplasm. The following are examples of such unknowns:

18. *The double-stranded RNA may get trapped in the nucleus.* It was known to those skilled in the art that multiple proteins interacted with single-stranded mRNA in the nucleus to mediate its translocation through the nuclear pore to the cytoplasm. It was possible that the canonical export machinery would not have recognized or bound to double-stranded RNA to facilitate its transport out of the nucleus. Alternatively, there may have been nuclear retention factors that could bind the duplex RNA to prevent it from leaving the nucleus. For example, Okano et al. reported that antisense RNA specific for the myelin basic protein formed duplexes in the nucleus and suggested that duplex formation inhibited transport of mRNA from the nucleus (Okano H. et al., J. Neurochem., 1991, attached hereto as **Exhibit D**). It was possible that double-stranded RNA produced in the nucleus could suffer the same fate.

19. *Double-stranded RNA produced in the nucleus may get modified.* Double-stranded RNA produced in the nucleus would be exposed to various nucleus-specific enzymes that could

modify the RNA. One example of such an enzyme is a nuclear double-stranded RNA dependent adenosine deaminase. In the nucleus, this enzyme targets the double-stranded RNA portions of duplexes and converts adenosine (A) to inosine (I), which makes the duplex unstable. The instability of the duplex may lead to its unwinding, which would expose the unwound strands to further causes of degradation (Kumar M and Carmichael G, Microbiol. Mol. Biol. Rev., 1998, attached hereto as **Exhibit E**). Thus, unwinding of the duplex would have destroyed the important double-stranded structure of the RNA, and would be thought to render the RNA incapable of mediating gene silencing. Additionally, the incorporation of inosine in the RNA would have decreased the stringency of the intramolecular base-pairing within the duplex, which could have resulted in a heterogeneous collection of imperfect duplexes and other aberrant RNA secondary structures in the nucleus. Because one of ordinary skill in the art could not predict the effect that inosines would have on the system reported by Fire et al., it would have been difficult to predict if inosine-containing RNA duplexes produced in the nucleus would function.

20. *Double-stranded RNA produced in the nucleus may get degraded.* At the time of the present invention, a major concern with introducing RNA into cells was the degradation of that RNA. Most of RNA degradation machinery in the cytoplasm was suspected to target single stranded RNA at the 5' or the 3' termini. Double stranded RNA in the cytoplasm was generally thought to be resistant to this type of degradation. However, production of double-stranded RNA in the nucleus exposed that RNA to specialized RNAses that targeted double-stranded RNA, such as RNAse III (Wu H et al., J. Biol. Chem., 1998, attached hereto as **Exhibit F**). These enzymes would specifically lead to the degradation of duplex RNA in the nucleus, but not of the duplex RNA directly introduced into the cytoplasm by Fire et al. It could not have been predicted whether duplex structures would be degraded in the nucleus, thus compromising the double-stranded RNA and the ability to specifically silence genes as reported by Fire et al.

21. *Polyadenylation at the 3' end of RNA may interfere with RNA interference.* Messenger RNA precursors (pre-mRNA) that are produced in the nucleus are modified at their 3' terminus by the addition of a polyadenylation signal (poly-A tail) of ~200-250 adenine residues (Lodish et al., Molecular Cell Biology, c1999, attached hereto as **Exhibit G**, summarizing knowledge prior to the priority date). At the time of the present invention, two proposed functions of the poly-A tail were: (1) to protect the transcript from degradation (Sachs A. and Wahle E. J. Biol. Chem. 1993, attached hereto as **Exhibit H**); and (2) to stimulate transportation out of the nucleus (Huang Y. and Carmichael G., Mol. Cell. Biol., 1996, attached hereto as **Exhibit I**). Therefore, it would have been presumed to be important for RNA produced in the nucleus to have a poly-A tail for protection and transport out of the nucleus. However, one skilled in the art would not have been able to predict the effect that a poly-A tail would have had on the ability of an RNA duplex to mediate the interference reported by Fire et al. The poly-A tail would lead to a large, single-stranded overhang on both strands of the RNA duplex produced in the nucleus. Because the RNA duplexes of Fire et al. did not have a poly-A tail, it was not possible to predict how this structure would affect the function of the RNA duplex.

22. *Heterogeneous nuclear ribonucleoproteins may affect double-stranded RNA formation.* When pre-mRNA is produced in the nucleus, it is quickly bound by numerous heterogeneous nuclear ribonucleoproteins (hnRNPs). One function of these proteins is to promote the correct processing of endogenous pre-mRNA by preventing folding of the RNA onto itself and the formation of secondary structures (Lodish et al., Molecular Cell Biology, c1999, attached hereto as **Exhibit G**). A linearized RNA molecule is more easily accessible to the factors responsible for 5' end capping, splicing, and 3' end polyadenylation. Thus, as RNA of the present invention was transcribed in the nucleus, hnRNPs would be thought to bind to the RNA and prevent it from base-pairing with its complementary strand. If the RNA was

maintained in a linear form, it may not have been able to promote the effects reported by Fire et al. with RNA that was introduced in a double-stranded form. Additionally, the linear RNA would have been more susceptible to single strand specific RNAses in the nucleus as well as in cytoplasm.

23. *Binding of heterogeneous nuclear ribonucleoproteins may inhibit RNA interference function.* Additionally, it was known that some hnRNPs from the nucleus remain associated with mRNA as it is translocated into the cytoplasm (Lodish et al., Molecular Cell Biology, c1999, attached hereto as **Exhibit G**). Since the mechanism of the interference reported by Fire et al. was unknown, it was not possible to predict at the time if proteins bound to the RNA would have an effect on its ability to cause gene silencing. Possible consequences of bound nuclear proteins are: (1) they could obstruct a cleavage site that might have been important; (2) they could obstruct a binding site or interaction domain for other proteins that played a role; (3) they could physically block other proteins from interacting with the RNA; or (4) they could interfere with a potential interaction between the RNA and its target. Thus, if the RNA was able to assume a duplex structure, but the nuclear proteins remained bound to the RNA duplex when it encountered the silencing targets or unknown effectors in the cytoplasm, the bound duplex may not have been able to function properly to cause silencing.

Changes to the RNA secondary structure

24. Fire et al. explained that the double-stranded structure of the RNA was critical to the reported effects; therefore, one of ordinary skill in the art could not predict how the manipulation of that structure would influence gene silencing. Possible alternative RNA structures with double-stranded features include hairpins/stem loops, pseudoknots, hammerheads and cloverleafs, each of which could be formed by a single strand of RNA. There is no

information in the Fire et al. Provisional or in the Fire et al. Nature publication which would allow one of ordinary skill to predict whether any of the other structures, and most importantly whether two separate complementary RNA strands, were required for activity. If two separate strands of RNA were needed for the RNA interference mechanism, for example, if the unknown mechanism required that the two strands separate *in vivo*, then one of ordinary skill would question whether a single, self-complementary strand would function. Alternatively, if two free ends of the RNA molecule were required for the unknown mechanism of the interference reported by Fire et al., then one would question whether a single self-complementary RNA strand would function. Thus, it could not be predicted at the time whether the hairpin RNA structure produced by the DNA constructs of the present invention would be able to cause the interference effect reported by Fire et al.

Changes to the size of the double stranded RNA

25. The shortest RNA with which Fire et al. observed interference was 299 base pairs long. Although the Fire Provisional discloses a range of RNA lengths from 25 to 400 base pairs corresponding to the target gene, Fire et al. neither suggest, nor demonstrate if any of the lengths encompassed by that range is optimal. Furthermore, it was totally unknown whether double-stranded RNA molecules shorter than 299 nucleotides could operate in RNA interference, as no results for such experiments had been reported at the time. The present invention teaches the use of DNA constructs that would produce a hairpin RNA having a duplex region 20-30 base pairs long. There was no indication that the use of RNAs an order of magnitude shorter than those shown to work by Fire et al. would have caused the same result. If a certain length of nucleotides within the double-stranded RNA was required for RNA interference, then small double-stranded RNA molecules below the minimum size requirement would not operate by the

unknown mechanisms of the system. Because the mechanism was not known, the size requirements were a mystery and one could not predict the consequence of decreasing the length of the RNA duplex.

Change to expressing double-stranded RNA in mammalian cells

26. At the time of Applicants' invention, there was widespread uncertainty in the art about whether double-stranded RNA could be delivered to mammalian cells without causing the cells to die. It was known in the art that double-stranded RNA caused the interferon response in mammalian cells which led to global mRNA degradation and translation inhibition (Reviewed in Jacobs B.L. and Langland J.O., Virology, 1996, attached hereto as **Exhibit J**). Because this response existed, it was impossible to predict whether double-stranded RNA would cause specific RNA interference in mammalian cells without triggering the non-specific double-stranded RNA response. Thus, any attempts to induce specific RNA interference in mammalian cells could not have been viewed as obvious due to the prevailing belief in the art at the time that RNA interference would not be possible if the double-stranded RNA response was functioning in the targeted mammalian cells.

27. The uncertainties imposed by deviating from the teachings of Fire et al. were significant. Consequently, it was not possible for one of ordinary skill in the art prior to Applicants' invention to predict if the invention claimed by Applicants would have succeeded in causing any interference effect. The Applicants' Provisional was the first to disclose that the specific combination of elements as claimed would cause interference. The Examiner's reliance on Cowsert et al., Agrawal et al., and Kool, which come from irrelevant arts, would not have alleviated the concerns of those of ordinary skill in the art which I have described above.

28. In conclusion, I believe that there is no information in the Fire et al. Provisional, the Fire et al. Patent, Cowsert et al., Agrawal et al., or Kool which would have permitted one of ordinary skill in the art to reasonably expect prior to the Applicants' invention that the specific combination of features of the Applicants' claimed invention would result in any interference. Specifically, Applicants' selection of structural gene regions with a targeting region of 20-30 nucleotides to produce double-stranded RNA in mammalian cells was not disclosed by Fire et al. and one of skill in the art at the time would not have expected such a combination to cause RNA interference, despite the disclosure of Fire et al. I firmly believe that the endogenous production of RNA as claimed for the purpose of inhibiting expression of the target gene was a novel and non-obvious invention at the time the Applicants filed their Provisional application, *i.e.* on March 20, 1998.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified patent.

Arthur D. Riggs, Ph.D.

9/20/09
Date